The actions, detection and synthesis of platelet-activating factor (PAF) in mammalian pregnancy

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Abstract Platelet-activating factor (PAF) exhibits a variety of biological activities and it be thought to involved in various pathophysiological process. In this paper, some studies were summarized about those roles of PAF in a variety productive processes of female of mammalian that include fertilization, implantation and parturition, and that was involved in the concentration, synthesis, degradation and some assay methods of PAF. The relationship between PAF and early pregnancy factor (EPF) was reviewed.
Key words: Platelet-activating factor, Mammalian, Pregnancy, Early pregnancy factor

Platelet-activating factor (PAF:1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) was discovered as a chemical mediator, released from sensitized basophils (Benveniste *et al* 1972), that caused platelet aggregation. Subsequent studies demonstrated that PAF was produced upon appropriate stimulation of a variety of human cells, including neutrophils, eosinophils, monocytes, and endothelial cells, and this potent lipid mediator is involved in a number of the allergic and inflammatory responses (Hanahan 1986; Braquet *et al* 1987; Wallace 1989; Prescott *et al* 1990). PAF is also found in spermatozoa (Kumar et al 1988) preimplanted embryos (O' Neill 1985), amniotic fluid (Billah 1983), and the uterus (Yasuda et al 1986; Angle et al. 1988). PAF is considered to be involved in a variety of reproductive processes including fertilization (Roudebush *et al* 1990), implantation (Ryan *et al* 1990), and parturition (Johnston 1989). The roles of PAF in parturition are not fully defined, but it is well documented that PAF induces myometrial contraction (Tetta *et al* 1986) and promotes the synthesis of prostaglandin E_2 (Billah *et al* 1985).

PAF in the establishment of pregnancy and ovoimplantation

PAF may be the first physiology signal produced by the embryo for maternal recognition of pregnancy. In mice and humans fertilization induces a pregnancy-associated thrombocytopenia (O'Neill 1985a) which has been attributed to release of platelet-activating factor (PAF) by the zygote (O'Neill 1985b, c). Ammit and O'Neill (1991) subsequently confirmed the release of embryonic PAF into culture media, and O'Neill and Saunders (1984) identified a correlation between media PAF concentration, embryo implantation rate and successful pregnancy.

PAF is produced by uterine tissue and pre-implantation-stage embryos. PAF level in the rabbit uterus increase from Day 3 to Day 5 of pregnancy (Angle et al 1988). Embryonic PAF production in the rabbit and mouse also increases during the pre-implantation phase, with maximum levels at the expanded blastocyst stage (Minhas *et al* 1993; Ripps *et al* 1993). PAF production by human embryos has been correlated with pregnancy potential (Nakatsuka et al., 1992; O'Neill *et al* 1987). Furthermore, mouse embryos cultured in the presence of PAF have enhanced developmental rates (Roberts *et al* 1993) and higher implantation rates upon transfer to synchronized recipients (Ryan *et al* 1987). Addition of exogenous PAF to the culture medium improves two-cell development to the blastocyst stage in the mouse (Nishi *et al* 1995; Roudebush *et al* 1996; O'-Neill 1997), apparently which may be due, in part, to embryo-derived PAF stimulation of embryonic metabolism (Ryan *et al* 1989). PAF directly influences the oxidative metabolism of glucose and lactate in the pre-implantation mouse embryo (Ryan *et al* 1990). These observations suggest that PAF production is a prerequisite for pregnancy, and that it could be used as a marker for embryo viability. The effect of PAF on in vitro mouse embryo development appears to be strain specific (Radonjic-Lazovic *et al* 1995). Enhanced embryo development has also been reported in rabbit oocytes fertilized in vitro with PAF-treated spermatozoa (Roudebush *et al* 1993).

PAF antibodies inhibit embryo development (Roudebush *et al* 1993), and antagonists inhibit im-

plantation (Spinks *et al* 1988; Andu et al 1990; Norris *et al* 1994). Providing further evidence on the presence and requirement of embryo-derived PAF during the pre-implantation period. It appears that PAF's action in the embryo may be receptor mediated, since various PAF antagonists competitively inhibit its action (Nishi *et al* 1995). Roudebush *et al* (1997) determine the presence of the PAF receptor in the mouse preimplantation-stage embryo, and exogenous PAF treatment of the two-cell-stage CFW mouse embryo resulted in a fourfold increase in intracellular calcium over background levels, calcium has proven to be an important second messenger that controls or influences a variety of cellular functions. PAF receptors have been described in both sites in other species (Yang *et al* 1991, 1992).

Velasquez *et al* (1995) found that the PAF antagonists TCV-309 and BN-52021 delayed significantly the transport of egg to the uterus in pregnant animals, but not in cycling animals; i.e., they retarded the passage of embryos but not of oocytes to the uterus. Administration of PAF to cycling hamsters hastened the oviductal transport of ova. These data suggest that in the hamster, the earlier passage of embryos to the uterus as compared to oocytes is mediated by PAF. The decidual-like reaction induced by PAF is observed by Acker *et al* (1989) in the rat uterus.

Taken together those all confirmed that PAF is an important mediator in the establishment of pregnancy and ovo-implantation which may lead to improvement in *in vitro* fertilization and open up the possibility that PAF antagonists may be used as local contraceptive agents.

The Relationship between PAF and EPF

Early pregnancy factor (EPF) is one of the earliest biochemical indicator of pregnancy(Morton *et al* 1974). It has been detected at about the time of the pronuclear stage of the embryo in all mammals investigated including mice (Morton *et al* 1974), human (Morton *et al* 1977), several domestic (Morton, *et al* 1979; Nancarrow *et al* 1981) and other species. The ability of EPF in sera of pregnant animals to act on lymphocytes to cause increased rosette inhibition titres in the rosette inhibition assay has been studied for a considerable time (Smart et al 1981; Whyte and Heap 1983; Morton 1984; Chard and Grudzinkas 1987; Morton *et al* 1987)

Orozco et al (1986) injected PAF into mature female mice at fourth stage of the oestrous cycle, mature male mice and immature female mice. Only animals that were in dioestrus, pro-oestrus or oestrus gave a positive EPF result within 1 h of injection. This group also demonstrated that PAF in combination with oestrous mouse serum could give a positive response in the rosette inhibition test (Orozco *et al* 1990). The susceptibility of EPF activity in sera of pregnant animals to ammonium sulfate fractionation is well known (Clarke *et al* 1980; Sueoka *et al* 1989). Clark *et al* (1994) reinvestigated the effects of ammouium sulfate fractionation. Treatment of sera from pregnant mice with 40% ammonium sulfate was shown to liberate low molecular mass active moieties from their association with macromolecular serum components. After centrifugation, these active moieties partition into the supernatant fraction, while the macromolecular components to which they are bound partition into the pellet fraction. The macromolecular components of the supernatant and pellet fraction freed of any association with these low molecular mass moieties by extensive dialysis, can not alone induce increased rosette inhibition titres. In combination, however, components in the dialyzed pellet retentate fraction, cooperate with components in the dialysed supernatant retentate fraction to allow the expression of increased rosette inhibition titres when applied to fresh spleen cell in the rosette inhibition assay. In two-step incubation protocols, a prescribed order of addition must be followed if this cooperative effect is to be observed, namely supernatant retentate fraction in the second step, but not vice versa (Clark *et al* 1994).

Orozco *et al* (1994) demonstrated that the supernatant retentate fractions derived from sera of pregnant mice were functionally equivalent to PAF, or a serum stimulus because they stimulated the spleen cells used in the assay to produce active moieties and cooperated with pure thioredoxin in allowing for expression of activity. Conversely, the pellet retentate fractions obtained from sera of pregnant mice were shown to be functionally equivalent to thioredoxin in that they cooperated with a PAF stimulus to allow for the expression of increased rosette inhibition titres. Lash *et al* (1997) confirmed that PAF could stimulate the production of EPF by oestrous mouse ovaries and oviducts in vitro. Taking together the results that it could be suggested that PAF may be a component part responsible for EPF activity or PAF has the ability to induce the synthesis of EPF.

Biosynthesis and Degradation of PAF

PAF concentration in tissues or plasma is governed by the equilibrium between its biosynthesis and degradation. Studies in various cell types indicate that PAF synthesis may occur via one of two biosynthetic pathways the 'de novo' and 'membrane remodeling' pathways(for review see Snyder 1990). The immediate precursors of PAF in the de novo pathways are 1-O-alkyl-2-acetyl-sn-glycerol (alkylacetylglycerol) and cytidinediphosphocholine. In the membrane re-

modeling pathway, the substrates are 1-O-alkyl-2-lyso-sn-glycero-3-phosphocholine (lyso PAF) and acetyl-coenzyme A (acetyl-CoA). These reactions are catalyzed by dithiothreitol-insensitive cytidinediphosphocholine: 1-O-alkyl-2-acetyl-sn-glycerol cholinephosphotransferase (EC 2.7.8.16, cholinephosphotransferase), and by acetyl-coenzyme A:1-O-alkyl-2-lyso-sn-glycero-3-phosphocholine acetyltransferase (EC 2.3.1.67, acetyltransferase), respectively(Snyder 1990). The net synthesis of PAF occurs via the de novo pathway. The remodeling pathway, on the other hand, plays a role in the acute inflammatory process (Wykle *et al* 1980; Renooij et al 1981). Preimplantation embryos of mice (O'Neill 1985), humans (Collier *et al* 1988, 1990) and sheep (Battye *et al* 1991) secrete platelet-activating factor, which is detected in culture media. Production of PAF by the embryo in culture media ranges from 4.8 to 385.8 ng PAF per-embryo per 24 h for human embryos created by in vitro fertilization, and from 0.8 to 16.9 ng PAF per embryos per 24 h for mouse embryos (Collier *et al* 1988). This high output suggests that pre-implantation embryos may produce PAF by synthesis de novo.

Wells and O'Neill (1994) successfully detected the PAF biosynthetic enzymes, cholinephosphotransferase and acetyl-transferase in extracts of oocytes, zygotes and per-implantation embryos. The results indicate that the enzymes that catalyze the final step in both biosynthetic pathway are present within the oocyte and early embryo, while the activation of acetyltransferase following fertilization indicates that this enzyme may be of regulatory significance in the initiation of biosynthesis of PAF by zygotes.

PAF is generally thought to be labile in vivo. Biologically active PAF is converted to the biologically inactive lyso-PAF by PAF-acetylhydrolase (PAF-AH). PAF-AH activity is decreased in maternal plasma in rabbits (Maki *et al* 1988), human (Johnston 1989), rat and in rat uterus, in contrast, the placental PAF-AH activity significantly increased during the later stages of pregnancy (Matsubara *et al* 1997). It has been suggested that PAF-AH may regulate, in part, the PAF concentration in plasma or tissue.

The Measure of PAF

PAF bioassay

Embryo-derived PAF was discovered by O'Neill (1985), using a splenectomized mouse bioassay. Briefly, medium or test substances were given as a single i. p. injection (300 ul) into splenectomized mice. Platelet counts were performed immediately before and 30 min after injection, animals were bled under light anaesthesia from the peri-orbital plexus and 10 ul blood were diluted into 2 ml ammonium oxalate (1% w/v). In each assay, samples of PAF-acether were run as positive controls.

Collier et al (1988) provides a quantitative rapid (15 min) method to measure PAF based on the determination of PAF-induced platelet aggregation. A platelet analyzer was used to count single platelet in a suspension of whole blood.

The another traditional methods for the detection and measurement of PAF rely on the release of tritiated serotonin (Hanahan and Weintraub 1985)

PAF radioimmuno assay

Smal believed that all those biological assays have problems of large variability within and between assays, and the need for careful control of non-specific interference which cause platelet activation. The development of a specific radioimmunoassay (RIA) for PAF (Smal *et al* 1990b, c) overcomes some of these problems, and allows accurate quantitation of low levels of PAF in biological samples (0.02-2 pmol).Briefly, the acidified sheep anti-PAF antiserum, the anti-sheeplg G, synthetic PAF standard or unknown acidified sample and the(^{125}I)-labelled PAF tracer were mixed together. The radioactivity was quantitated by RIA. The PAF concentration was counted.

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(Responsible Editor: Chai Ruihai)